**Citation:**

Ballesteros MN, Cabrera RM, Saucedo Mdel S, Fernandez ML. Dietary cholesterol does not increase biomarkers for chronic disease in a pediatric population from northern Mexico. *Am J Clin Nutr.* 2004 Oct;80(4):855-61.

**PubMed ID:** 15447890

**Study Design:**

Randomized Crossover Trial

**Class:**

A - Click here for explanation of classification scheme.

**Research Design and Implementation Rating:**

POSITIVE: See Research Design and Implementation Criteria Checklist below.

**Research Purpose:**

To evaluate the effects of dietary cholesterol provided by whole eggs on plasma lipids and LDL atherogenicity in a pediatric population (children eight to 12 years of age) from a region in Mexico where significant dyslipidemias were identified in adults.

**Inclusion Criteria:**

- Children eight to 12 years of age
- Attendance of parents at informational meetings
- Signed parental consent form.

**Exclusion Criteria:**

- Children younger than eight years or older than 12 years of age
- No signed parental consent form.

**Description of Study Protocol:**

**Recruitment:** Recruited from the school Mauricio Kelly in Hermosillo, Mexico

**Design:** Randomized crossover trial

**Blinding used (if applicable):** Subjects were blinded to intervention

**Intervention:**

- Children were divided into two groups and randomly assigned to either the egg or egg substitute intervention for 30 days followed by a three week washout period and then allocation to the other intervention for 30 days.
- The children consumed either two whole eggs (providing 518 mg additional dietary

cholesterol) or the equivalent amount of egg whites with added color.
- Both the egg and substitute product were served as scrambled eggs to all children for breakfast in the school cafeteria.
- Eggs were packed for consumption on the weekend and parents were instructed on proper administration of the product.

**Statistical Analysis**

- Student's t test used to compare initial characteristics between genders
- Two-way analysis of variance (ANOVA) used to analyze initial plasma lipids in boy and girl hyperresponders and hyporesponders
- Repeated measures ANOVA used to analyze diet effects, responder effects and the interactions for plasma lipids, apolipoproteins, dietary components, distribution of cholesterol in LDL subfractions and LDL peak size.

**Data Collection Summary:**

**Timing of Measurements**

- Fasting blood samples to measure plasma lipid levels were collected on two different days at the beginning of the study and at the end of each diet treatment and washout period
- Weight, blood pressure and pedometer measured level of activity were measured at baseline and after each treatment period
- Three day weighed food record completed by children and parents during both treatment periods
- Lipoprint LDL system used to determine LDL peak particle diameter and subclass distribution

**Dependent Variables**

- Change in lipoprotein profile measured using blood samples collected on two different days
- Change in LDL subfractions and LDL peak diameter

**Independent Variables**

- Dietary cholesterol intake
- Children were divided into two groups and randomly assigned to either the egg or egg substitute intervention for 30 days followed by a three week washout period and then allocation to the other intervention for 30 days.

**Control Variables**

- Body mass index
- Diet composition evaluated by three day weighed foods records
- Level of physical activity
- Blood pressure
- Age.

**Description of Actual Data Sample:**

**Initial N**: 60 children (30 boys, 30 girls)

**Attrition (final N):** 54 children (25 boys, 29 girls)

**Age**: Boys 10.6 ± 1.6 years, Girls 10.2 ± 1.5 years

**Ethnicity**: Mexican

**Other relevant demographics**: Subjects attended a school located in one of the lowest socioeconomic quarters of the city of Hermosillo, Mexico

**Anthropometrics:**

**Location**: Hermosillo, Mexico

**Summary of Results:**

**Key Findings**

- Subjects classified as:
  - hyporesponders (no increase or < 0.5 mmol/L increase in plasma cholesterol for 100 mg dietary cholesterol)
  - hyperrespsonders (>0.6 mmol/L increase in plasma cholesterol for 100 mg dietary cholesterol)
- During the EGG period, the hyperresponders (n=18) had an elevation in both LDL cholesterol (from 1.54 ± 0.38 to 1.93 ± 0.36 mmol/L) and HDL cholesterol (from 1.23 ± 0.26 to 1.35 ± 0.29 mmol/L) with no changes in LDL:HDL.
- In contrast, hyporesponders (n=36) had no significant alterations in plasma LDL or HDL cholesterol.
- All subjects had an increase in LDL peak diameter during the EGG period (P < 0.01) and a decrease (P < 0.01) in the smaller LDL subfractions.

| | LDL Cholesterol mmol/L | HDL Cholesterol mmol/L | Triacylglycerol mmol/L | Cholesterol:HDL mmol/L | Apolipoprotein B mg/L |
|---|---|---|---|---|---|
| Hyperresponders | | | | | |
| Egg | 1.93±0.36 | 1.35±0.29 | 1.00±0.68 | 2.85±0.57 | 606±96 |
| Substitute | 1.54±0.38 | 1.23±0.26 | 1.02±0.37 | 2.66±0.52 | 598±126 |
| Hyporesponders | | | | | |
| Egg | 1.88±0.42 | 1.28±0.19 | 0.93±0.31 | 2.96±0.55 | 583±106 |
| Substitute | 1.83±0.44 | 1.22±0.19 | 1.10±0.47 | 3.00±0.53 | 627±109 |
| Diet Effect | P<0.0001 | P<0.001 | Not significant | Not significant | Not significant |
| Responder Effect | P<0.001 | P<0.001 | Not significant | Not significant | Not significant |

| | | | | | |
|---|---|---|---|---|---|
| Interaction | P<0.01 | P<0.05 | Not significant | Not significant | Not significant |

- Total cholesterol:HDL and LDL:HDL, markers of coronary heart disease risk, were maintained during both treatment periods for all subjects.
- Plasma apo C-III and apo-E concentrations did not change during either period.

| | LDL-1<br>mmol/L | LDL-2<br>mmol/L | LDL-3<br>mmol/L | LDL peak diameter<br>nm |
|---|---|---|---|---|
| Hyperresponders<br>Egg<br>Substitute | 1.52±0.57<br>1.16±0.29 | 0.34±0.19<br>0.27±0.13 | 0.16±0.23<br>0.26±0.28 | 26.51±0.10<br>26.10±0.11 |
| Hyporesponders<br>Egg<br>Substitute | 1.45±0.49<br>1.37±0.42 | 0.40±0.22<br>0.39±0.14 | 0.24±0.23<br>0.25±0.24 | 26.32±0.09<br>26.19±0.09 |
| Diet Effect | P<0.01 | Not significant | P<0.05 | P<0.01 |
| Responder Effect | Not significant | Not significant | Not significant | Not significant |
| Interaction | Not significant | Not significant | Not significant | Not significant |

- Of 54 children, 34 (63%) presented the pattern associated with small dense LDL (LDL-3) during egg substitute intervention. Five children shifted from B to A during the egg intervention period.
- Between boys and girls, no significant differences were seen in age, plasma total cholesterol, LDL cholesterol, HDL cholesterol, triacylglycerol, level of activity or blood pressure at baseline. BMI was significantly higher among boys, 20.9±4.3, as compared to girls, 18.4±2.9, with P<0.05.
- No significant differences were seen in activity level, systolic blood pressure, or BMI for hyporesponders or hyperresponders during the egg or substitute period. The diastolic blood pressure was lower during the egg period for both hyporesponders and hyperresponders (diet effect, P<0.05).
- Population consumed a high fat diet independent of dietary period.
- Total calories consumed were not different for hyporesponders or hyperresponders during both periods
- Dietary cholesterol intake significantly higher for all subjects during egg period and can be mostly contributed to cholesterol content of egg yolks (P<0.001).
- Saturated fatty acid content was not significantly different for hyperresponders between diets. SFA intake was significantly lower during the egg substitute period for hyporesponders than in hyporesponders during the egg period or than the hyperresponders during either period (P<0.05).

**Author Conclusion:**

Intake of 2 eggs per day results in the maintenance of LDL:HDL cholesterol ratio and in the generation of a less atherogenic LDL in this population of Mexican children.

***Reviewer Comments:***

- *Parental consent, meeting attendance and involvement required for child to participate in study.*
- *Sponsored by the American Egg Board*

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***Research Design and Implementation Criteria Checklist: Primary Research***

| **Relevance Questions** | | |
|---|---|---|
| 1. | Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (Not Applicable for some epidemiological studies) | Yes |
| 2. | Did the authors study an outcome (dependent variable) or topic that the patients/clients/population group would care about? | Yes |
| 3. | Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to nutrition or dietetics practice? | Yes |
| 4. | Is the intervention or procedure feasible? (NA for some epidemiological studies) | Yes |

| **Validity Questions** | | |
|---|---|---|
| **1.** | **Was the research question clearly stated?** | Yes |
| 1.1. | Was (were) the specific intervention(s) or procedure(s) [independent variable(s)] identified? | Yes |
| 1.2. | Was (were) the outcome(s) [dependent variable(s)] clearly indicated? | Yes |
| 1.3. | Were the target population and setting specified? | Yes |
| **2.** | **Was the selection of study subjects/patients free from bias?** | Yes |
| 2.1. | Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study? | Yes |
| 2.2. | Were criteria applied equally to all study groups? | Yes |
| 2.3. | Were health, demographics, and other characteristics of subjects described? | Yes |
| 2.4. | Were the subjects/patients a representative sample of the relevant population? | Yes |

| | | |
|---|---|---|
| **3.** | **Were study groups comparable?** | Yes |
| 3.1. | Was the method of assigning subjects/patients to groups described and unbiased? (Method of randomization identified if RCT) | Yes |
| 3.2. | Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline? | Yes |
| 3.3. | Were concurrent controls used? (Concurrent preferred over historical controls.) | N/A |
| 3.4. | If cohort study or cross-sectional study, were groups comparable on important confounding factors and/or were preexisting differences accounted for by using appropriate adjustments in statistical analysis? | N/A |
| 3.5. | If case control or cross-sectional study, were potential confounding factors comparable for cases and controls? (If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies.) | N/A |
| 3.6. | If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g., "gold standard")? | N/A |
| **4.** | **Was method of handling withdrawals described?** | Yes |
| 4.1. | Were follow-up methods described and the same for all groups? | Yes |
| 4.2. | Was the number, characteristics of withdrawals (i.e., dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group? (Follow up goal for a strong study is 80%.) | Yes |
| 4.3. | Were all enrolled subjects/patients (in the original sample) accounted for? | Yes |
| 4.4. | Were reasons for withdrawals similar across groups? | N/A |
| 4.5. | If diagnostic test, was decision to perform reference test not dependent on results of test under study? | N/A |
| **5.** | **Was blinding used to prevent introduction of bias?** | Yes |
| 5.1. | In intervention study, were subjects, clinicians/practitioners, and investigators blinded to treatment group, as appropriate? | Yes |
| 5.2. | Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.) | Yes |
| 5.3. | In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded? | N/A |
| 5.4. | In case control study, was case definition explicit and case ascertainment not influenced by exposure status? | N/A |

| | | |
|---|---|---|
| 5.5. | In diagnostic study, were test results blinded to patient history and other test results? | N/A |
| **6.** | **Were intervention/therapeutic regimens/exposure factor or procedure and any comparison(s) described in detail? Were interveningfactors described?** | Yes |
| 6.1. | In RCT or other intervention trial, were protocols described for all regimens studied? | Yes |
| 6.2. | In observational study, were interventions, study settings, and clinicians/provider described? | N/A |
| 6.3. | Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect? | Yes |
| 6.4. | Was the amount of exposure and, if relevant, subject/patient compliance measured? | Yes |
| 6.5. | Were co-interventions (e.g., ancillary treatments, other therapies) described? | Yes |
| 6.6. | Were extra or unplanned treatments described? | Yes |
| 6.7. | Was the information for 6.4, 6.5, and 6.6 assessed the same way for all groups? | Yes |
| 6.8. | In diagnostic study, were details of test administration and replication sufficient? | N/A |
| **7.** | **Were outcomes clearly defined and the measurements valid and reliable?** | Yes |
| 7.1. | Were primary and secondary endpoints described and relevant to the question? | Yes |
| 7.2. | Were nutrition measures appropriate to question and outcomes of concern? | Yes |
| 7.3. | Was the period of follow-up long enough for important outcome(s) to occur? | Yes |
| 7.4. | Were the observations and measurements based on standard, valid, and reliable data collection instruments/tests/procedures? | Yes |
| 7.5. | Was the measurement of effect at an appropriate level of precision? | Yes |
| 7.6. | Were other factors accounted for (measured) that could affect outcomes? | Yes |
| 7.7. | Were the measurements conducted consistently across groups? | Yes |
| **8.** | **Was the statistical analysis appropriate for the study design and type of outcome indicators?** | Yes |
| 8.1. | Were statistical analyses adequately described and the results reported appropriately? | Yes |
| 8.2. | Were correct statistical tests used and assumptions of test not violated? | Yes |

| | | | |
|---|---|---|---|
| | 8.3. | Were statistics reported with levels of significance and/or confidence intervals? | Yes |
| | 8.4. | Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)? | N/A |
| | 8.5. | Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g., multivariate analyses)? | Yes |
| | 8.6. | Was clinical significance as well as statistical significance reported? | Yes |
| | 8.7. | If negative findings, was a power calculation reported to address type 2 error? | N/A |
| **9.** | **Are conclusions supported by results with biases and limitations taken into consideration?** | | Yes |
| | 9.1. | Is there a discussion of findings? | Yes |
| | 9.2. | Are biases and study limitations identified and discussed? | No |
| **10.** | **Is bias due to study's funding or sponsorship unlikely?** | | No |
| | 10.1. | Were sources of funding and investigators' affiliations described? | Yes |
| | 10.2. | Was the study free from apparent conflict of interest? | No |